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Bioengineered bioluminescent magnetotactic bacteria as a powerful tool for chip-based whole-cell biosensors†

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This paper describes the generation of genetically engineered bioluminescent magnetotactic bacteria (BL-MTB) and their integration into a microfluidic analytical device to create a portable toxicity detection system. *Magnetospirillum gryphiswaldense* strain MSR-1 was bioengineered to constitutively express a red-emitting click beetle luciferase whose bioluminescent signal is directly proportional to bacterial viability. The magnetic properties of these bacteria have been exploited as "natural actuators" to transfer the cells in the chip from the reaction to the detection area, optimizing the chip's analytical performance. A robust and cost-effective biosensor for the evaluation of sample toxicity, named MAGNETOX, based on lens-free contact imaging detection, has been developed. A microfluidic chip has been fabricated using multilayered black and transparent polydimethyl siloxane (PDMS) in which BL-MTB are incubated for 30 min with the sample, then moved by microfluidics, trapped, and concentrated in detection chambers by an array of neodymium–iron–boron magnets. The chip is placed in contact with a cooled CCD *via* a fiber optic taper to perform quantitative bioluminescence imaging after addition of luciferin substrate. A model toxic compound (dimethyl sulfoxide, DMSO) and a bile acid (taurochenodeoxycholic acid, TCDCA) were used to investigate the analytical performance of the MAGNETOX. Incubation with DMSO and TCDCA drastically reduces the bioluminescent signal in a dose-related manner. The generation of bacteria that are both magnetic and bioluminescent combines the advantages of easy 2D cell handling with ultra sensitive detection, offering undoubted potential to develop cell-based biosensors integrated into microfluidic chips.

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Introduction

The increasing demand for robust and cost-effective portable analytical devices for on-site environmental toxicity screening has prompted the development of miniaturized whole-cell biosensors which are able to provide information about potential *in vivo* toxicity, thus predicting risks for human and animal health.[1] Advances in molecular biology techniques offer the opportunity to enable cells to express specific recognition elements such as receptors or regulatory proteins which trigger intracellular signaling events as a result of a specific interaction with target analyte(s). Reporter gene technology is based on a cascade of signaling events which ultimately result in the expression of a reporter protein, such as green fluorescent protein (GFP) or luciferase, whose expression can be measured by fluorescence or bioluminescence (BL).[2] Thanks to the high signal/noise ratio and no need for an external light source or special sample geometry, measurement of BL represents one of the most powerful detection strategies for miniaturized devices.[3] Besides, the availability of several luciferases with different BL emission properties allows development of cell-based BL assays in multiplex formats or use of an internal control to correct the analytical signal according to cell viability.[4] Whole-cell biosensors are suitable for implementation in miniaturized and/or microfluidic devices, with the advantages of low sample and reagent consumption, portability, and short analysis time. Several examples of detection of different classes of analytes, ranging from heavy metals to endocrine disruptors have been reported.[5,6] Nonetheless, the majority of previously reported devices lack adequate analytical performance for real-life applications, and have thus failed to reach the market.[7,8] To increase the robustness of such devices, several cell immobilization strategies have been investigated, aiming to keep the

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cells alive and responsive to the target analyte. However, analyte and/or reagent (*e.g.*, oxygen, BL substrates…) diffusion through the immobilization matrix may result in prolonged analysis time and reproducibility issues.

A promising strategy to improve the sensitivity of BL-based devices is to move the cells between different areas of the chip which are appointed to carry out specific functions such as: incubation of the sample, reagent(s) addition, washing and detection. To this end the exploitation of magnetotactic bacteria (MTB) magnetism as a "natural actuator" could represent a valuable approach.

MTB have the innate ability to produce magnetosomes (or bacterial magnetic nanoparticles, BacMPs), *i.e.* nanoparticles of magnetite (Fe_3O_4) or greigite (Fe_3S_4) enveloped in a 3–4 nm thick lipid membrane, which are aligned in a well-ordered chain to achieve the maximum magnetic moment. Owing to the presence of magnetosomes, MTB orient and migrate along geomagnetic field lines. In past years, extensive research has been focused on the elucidation of the mechanisms regulating magnetosome biosynthesis and on the optimization of culture techniques for the production and purification of BacMPs.[9,10]

Two major strategies can be envisaged to exploit MTB for bioanalytical applications: (i) the use of genetically engineered MTB as living biosensors, and (ii) the use of BacMPs as an alternative to chemically synthesized magnetic nanoparticles (MNPs). So far, the latter has been explored more extensively. Thanks to their size, ranging from 30 to 120 nm,[11] and their biocompatibility, due to the presence of a surrounding lipid bilayer membrane, BacMPs are highly advantageous for developing several kinds of biosensors, and more generally for binding assays[12,13] and drug delivery systems.[14]

Although significant advances in genomic studies of MTB have been reported,[15] the genetic engineering of MTB necessary to obtain magnetic bioluminescent whole-cell biosensors (BL-MTB) has not been accomplished. In addition, a miniaturized device integrating BL-MTB and a light detector has not been reported in the scientific literature.

As a first proof of concept of this new approach, *M. gryphiswaldense* MSR-1 strain was genetically engineered to constitutively express a red-emitting luciferase. A microfluidic chip prototype has been fabricated using multilayered polydimethylsiloxane (PDMS) constituted of three diamond-shaped incubation chambers connected with detection areas. After incubation with the sample, bacteria can be magnetically trapped and concentrated in the detection areas which are placed in contact with a charge-coupled device (CCD) sensor *via* a fiber optic taper to maintain adequate spatial resolution. Quantitative BL imaging is performed over a period of a few minutes, upon substrate addition (D-luciferin). This prototype was used as a rapid and sensitive biosensor for the evaluation of sample toxicity.

Results and discussion

In this study we genetically engineered magnetotactic bacteria to obtain BL magnetic biosensors. Some so-called "magnetically labeled biosensors" have been previously obtained by chemical functionalization of cells (mammalian cell lines, yeast and bacteria) with synthetic MNPs.[16,17] Indeed this approach has some technical limitations requiring functionalization steps that may create standardization problems; the cells divide during the incubation time and the number of MNPs on each cell's surface decreases, thus negatively affecting the reproducibility of an assay relying on the movement or trapping of biosensing cells within a microfluidic chip *via* magnetic fields. Our strategy exploits the intrinsic capability of magnetotactic bacteria, such as *Magnetospirillum gryphiswaldense*, to produce a chain of magnetic nanoparticles (magnetosomes) within the cell which confer the ability to orient and migrate along magnetic field lines. The number and shape of the genetically encoded magnetic particles is strictly regulated by the bacterial genome.

When grown in microaerobic conditions the majority of the cells are magnetic and retain magnetic properties through cell divisions. For the purpose of microfluidic chip integration we choose magnetotactic bacteria in order to have a homogenous population of magnetic cells.

The biosensors were integrated into a newly designed magnetic microfluidic chip, named MAGNETOX, which facilitates rapid, sensitive and direct toxicity screening without the need for sample pre-treatment steps.

The use of a magnetic array to separate the biosensing cells from the sample allows simultaneous removal of interferents and concentration of the BL-MTB in front of the CCD device, improving BL detection in terms of light output and sensitivity.

Preparation and characterization of bioluminescent magnetotactic bacteria

M. gryphiswaldense has been selected as the host strain because it is well characterized and its genetic toolbox is well developed.[18]

The *M. gryphiswaldense* strain MSR-1 was genetically engineered to express the red-emitting click beetle luciferase (CBR, λ_{max} = 615 nm) under the control of the constitutive $P_{mamDC45}$ minimal promoter which ensures high expression levels of heterologous proteins (unpublished data). To this end, CBR luciferase, a mutant of a yellow-green luciferase from *Pyrophorus plagiophthalamus*, was selected as the reporter protein because of its thermostability, pH-insensitivity and glow type emission.[19]

When expressed in bacterial cells, CBR has a much longer half-life than the wild-type *P. pyralis* luciferase (5 h *vs.* 0.26 h).[20] Since CBR requires endogenous bacterial ATP for the chemical reaction, any change in light output truly reflects alterations in the viability and metabolic state of the cell. A shuttle vector containing the cDNA encoding for CBR under the regulation of a constitutive promoter was used to transform an *E. coli* donor strain and was transferred to MSR-1 strain by conjugation.

The BL emission of the BL-MTB cells was investigated in terms of kinetics and spectral emission properties to obtain

information useful for the subsequent implementation of the cells in the microfluidic chip. For this reason we measured the BL signals emitted by intact living cells in 96-well microplates after addition of the BL substrate D-luciferin at pH 5.0. The BL-MTB emission kinetics showed that after injection of the BL substrate the signal reached a peak within a few seconds followed by rapid decay and stabilization at a signal corresponding to about 50% of the maximum BL emission (see Fig. 1(a)). Therefore a suitable temporal window from 5 to 15 min was identified for the BL measurements. This glow-type emission is particularly interesting for imaging applications since it allows integration of the BL signal over several minutes, thus increasing sensitivity. The BL emission spectrum showed a λ_{max} at 615 nm with a half bandwidth of 53 nm (see Fig. 1(b)), consistent with CBR expression in other bacterial systems such as *E. coli* strains (data not shown).

As known from other proteins previously expressed in MSR, cytoplasmic expression of the luciferase enzyme is not likely to influence magnetosome formation. This was confirmed by transmission electron microscope (TEM) characterization which revealed that the magnetosome number and morphology in BL-MTB was indistinguishable from those of MSR-1 wild-type strain (see Fig. 1(d)).

Investigation of the time dependent luciferase expression and cell magnetism led to 3 day-old cultures being selected for inclusion in the MAGNETOX biosensor. Fig. 1(c) shows both the BL signal and the cell magnetism (C_{mag}) of BL-MTB cultured in a medium containing Fe(III) citrate. Luciferase BL reached a maximum intensity at 72 h after inoculation, when micro-aerobic conditions prevailed and cell magnetism was 1.40 ± 5% (corresponding to 90% of the maximum C_{mag} obtained after 6 days incubation). Magnetic properties were also macroscopically confirmed by moving the BL-MTB with a permanent magnet (see movie M1†).

Design and fabrication of the MAGNETOX microfluidic chip

Exploiting multilayer PDMS casting, a microfluidic chip comprising incubation and detection chambers, in which BL-MTB can be loaded and trapped into specific positions for analysis, has been fabricated. The chip comprises three, 60 µl volume, diamond-shaped incubation chambers, each of them connected to two detection areas to which the BL-MTB can be navigated *via* a microfluidic system, then trapped and accumulated using permanent magnets (see Fig. 2). Subsequently the BL substrate can be delivered to the 6 detection chambers allowing simultaneous imaging. Lens-free BL

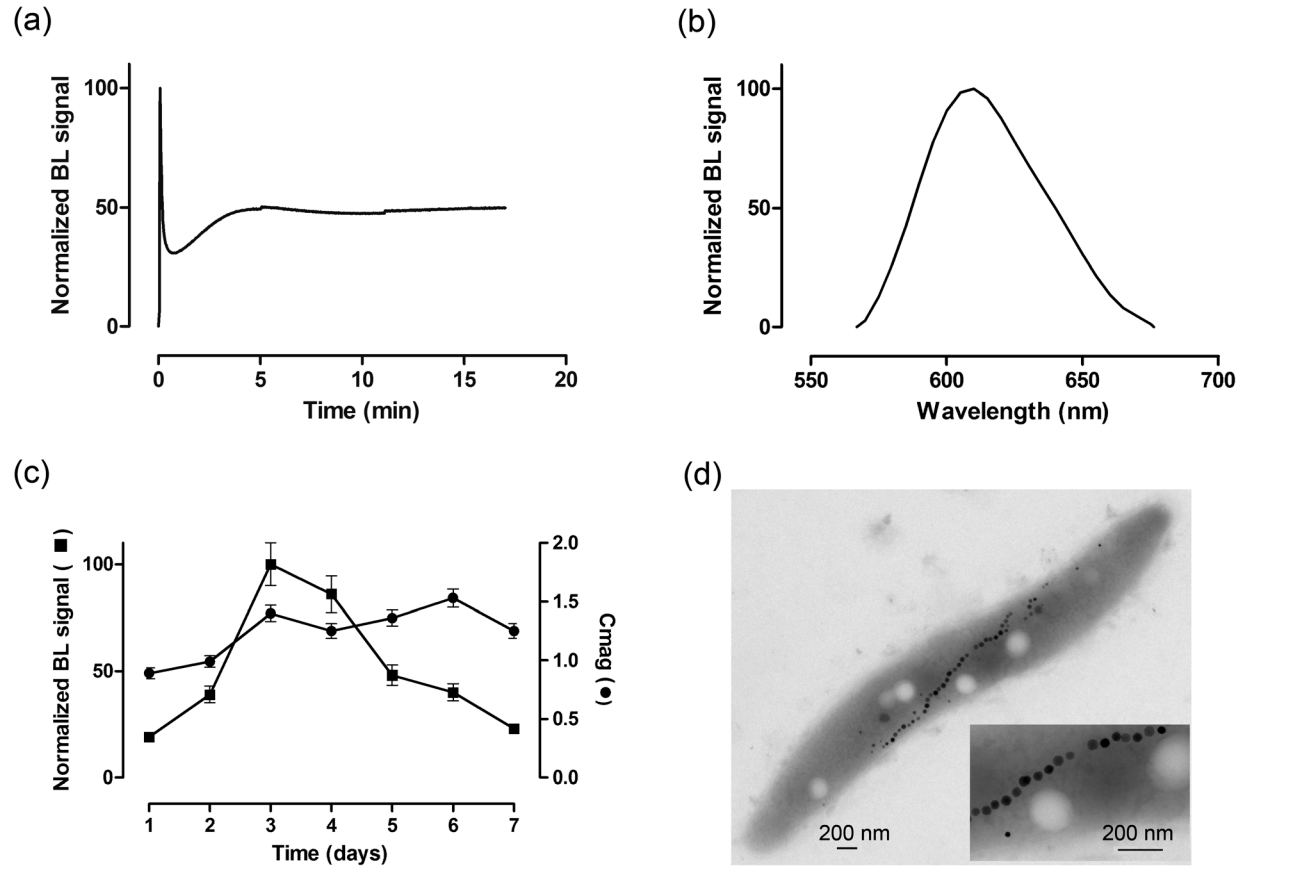

Fig. 1 Characterization of the bioluminescent magnetotactic bacteria BL-MTB. (a) Normalized BL emission kinetics. (b) Normalized emission spectrum (λ_{max} = 615 nm). (c) Time dependent expression of the CBR luciferase and cell magnetism (C_{mag}) of the growing BL-MTB cultures. (d) TEM micrographs of MSR-1 expressing CBR luciferase taken with a Morgagni 268 at 80 kV. Inset shows a magnified image of the magnetosome chain.

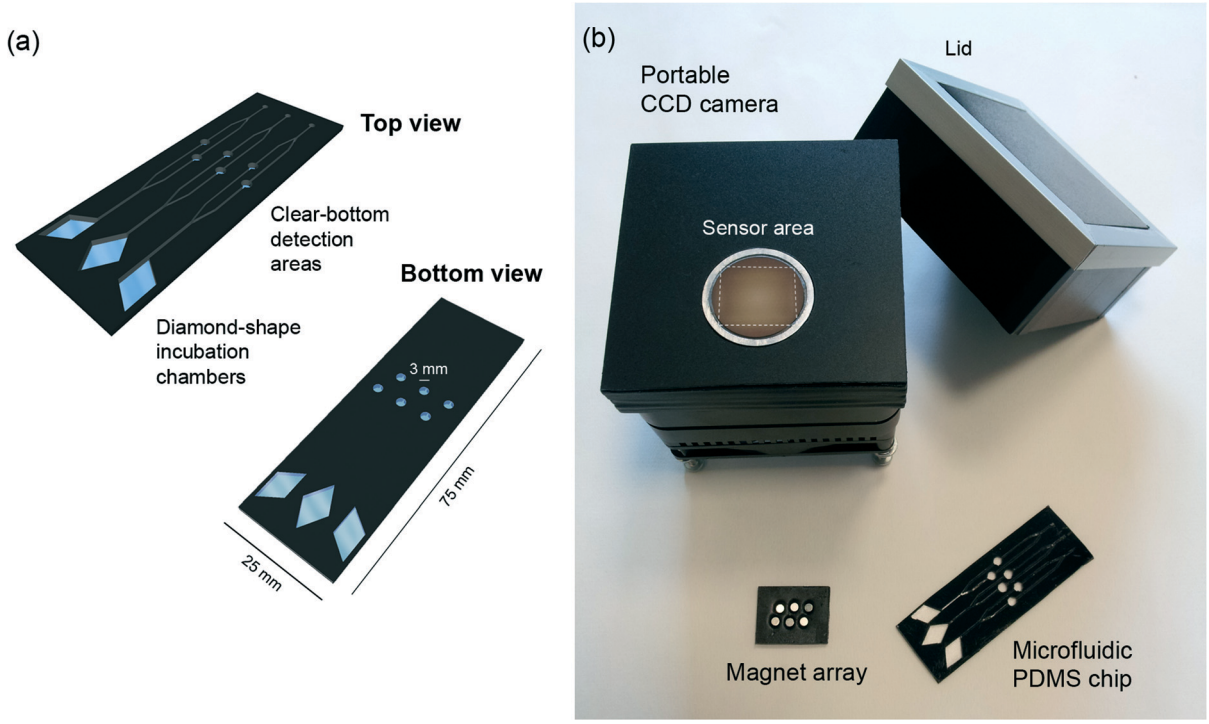

Fig. 2 The MAGNETOX biosensor. (a) Schematic representation of the microfluidic chip comprising three diamond-shape incubation chambers and clear-bottom detection areas. (b) Main components of the MAGNETOX biosensor: the microfluidic chip, the magnet array, the portable CCD camera with a fiber optic taper for lens-free BL imaging, and a lid for shielding against ambient light.

imaging was performed using a CCD camera interfaced with a fiber optic taper to maintain adequate sample resolution.[21] This prototype configuration was designed to concentrate the cells in a reduced volume, thus improving light collection from the detection chambers. This strategy will be pursued to further miniaturize the MAGNETOX chip and increase the number of detection areas.

Another feature of the MAGNETOX is that, after analysis, the cells are washed out of the chip so that it can be re-used. Since incubation of cells with the analyte lasts only 30 min no sterility is required and the chip can simply be washed with 70% ethanol before reuse. This makes the device suitable for applications in low-resource settings.

As we previously reported, CCD contact lens-less imaging does not require any optical system, thus simplifying the fabrication of compact and miniaturized analytical devices.[22] In addition lens-less imaging achieves a higher level of detection by taking advantage of the light collection efficiency of the system.

The Sony ICX285 monochrome CCD sensor has been selected since it has a quantum efficiency higher than 50% in the range of 420–680 nm which covers the whole emission of the CBR reporter. A fiber optic mosaic taper which transmits the emitted light directly to the CCD sensor was used to increase the sensing surface by 2.3 times compared to the actual size of the CCD sensor (*i.e.*, from 9.0 × 6.7 mm^2 to 20.7 × 15.4 mm^2), whilst still maintaining good resolution. A double Peltier cooling system reduced the thermal noise of the CCD, thus improving the signal/noise ratio and a lid provided shielding against ambient light.

The most significant improvements made to the device are: i) the implementation of a straightforward strategy to cast black and transparent PDMS; ii) the integration of a magnet array into the chip; iii) the design and fabrication of a microfluidics platform optimized for magnetic biosensors.

Although the use of black PDMS (a suspension of charcoal in PDMS) to fabricate microfluidic devices has been already reported elsewhere,[23] to the best of our knowledge the present work represents the first proof of concept for a black PDMS microfluidic chip that includes detection chambers with transparent bottoms for BL imaging detection. In addition this approach may have a more general application for optical detection.

We first investigated the ability of neodymium–iron–boron (NdFeB) magnets to rapidly and efficiently trap BL-MTB within the chip. Fig. 3(a) shows BL images of the MAGNETOX chip obtained after D-luciferin addition. At first, the signal is homogeneously distributed in the incubation chambers. As expected, after magnetic trapping, the BL signal is mostly concentrated in the detection chambers (approximately 85% of total light emission) (see Fig. 3(b)). Weak signals still appear along the microfluidic channels, indicating that the magnetic trapping could be further optimized to improve reproducibility. The prototype was purposely designed to disregard the signal emitted by BL-MTB in the microfluidic channels or in the incubation chambers. In fact the background signal of the non-trapped cells does not interfere with the CCD detection since the channels are made from black PDMS.

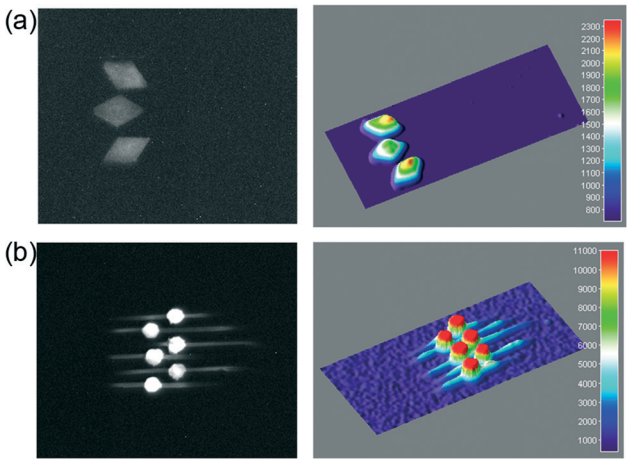

Fig. 3 BL images of BL-MTB inside the incubation chambers (a) and after cell movement and magnetic trapping in the detection chambers (b). The MAGNETOX chip was imaged from the top using a Night Owl LB 981 luminograph (EG&G Berthold, Bad Wildbad, Germany) with 5 min integration. A 3D surface plot visualization was obtained using ImageJ software.

In addition, thanks to the reflective surface of the magnets, BL emitted towards the opposite side of the sensor can also be detected, thus increasing the sensitivity of the analysis. The use of a reflecting surface increases the light signal by approximately 30% compared to that obtained with black tape covered magnets (data not shown).

Evaluation of sample toxicity with the MAGNETOX platform

The feasibility of the MAGNETOX platform as a general toxicity biosensor was assessed using standard solutions of DMSO, as model toxic compound and TCDCA, a detergent bile acid that causes structural and dynamic effects in membranes. The toxic effect of DMSO is mainly due to its activity in the cell membrane,[24] which affects cell metabolism and viability. The alteration of cell energy systems dramatically alters intracellular ATP levels, which can be monitored with an ATP-dependent luciferase. Therefore only metabolically active cells are able to provide the bioluminescent signal, which reflects their viability.

Concentration-response curves for DMSO were obtained and compared to those obtained for BL-MTB in a miniaturized 6-well cartridge (see Fig. S1(a)†). This microwell cartridge, containing 6 wells of 60 μL volume each, was purposely designed for integration in the same portable device. This allowed the actual advantage of concentrating BL-MTB *via* microfluidics and adding the BL substrate after a washing step to be assessed in comparison to a conventional microwell format using the same CCD detector. The BL recombinant cells were incubated for 30 min at room temperature with different concentrations of toxic compounds inside the MAGNETOX chip or inside the microwells. For the MAGNETOX assay, after incubation the cells were moved towards the detection chamber, and trapped by the magnetic array. Upon addition of the D-luciferin substrate, images were acquired with the CCD detector and analyzed to quantify BL emission (see Fig. 4(a)). Fig. 4(b) shows that the BL signal is strongly affected by DMSO with an LC_{50} of 7.5% v/v DMSO obtained in both configurations. The two curves showed a similar sensitivity with slight differences at low DMSO concentrations (*e.g.*, in the MAGNETOX a 2.5% v/v DMSO produces a signal of 75 ± 12% whereas 85 ± 5% is recorded in the microwell configuration) while at DMSO concentrations higher than 40% v/v complete cell death is observed in both the configurations.

We then used the bile acid TCDCA (0.001–10 mM). An increasing toxic effect was observed (see Fig. 5) while approaching the TCDCA critical micelle concentration (CMC 3 mM).[25] Micelles can modify the membrane constituents, thus resulting in a change in physicochemical properties causing adverse effects on cell viability.[26,27]

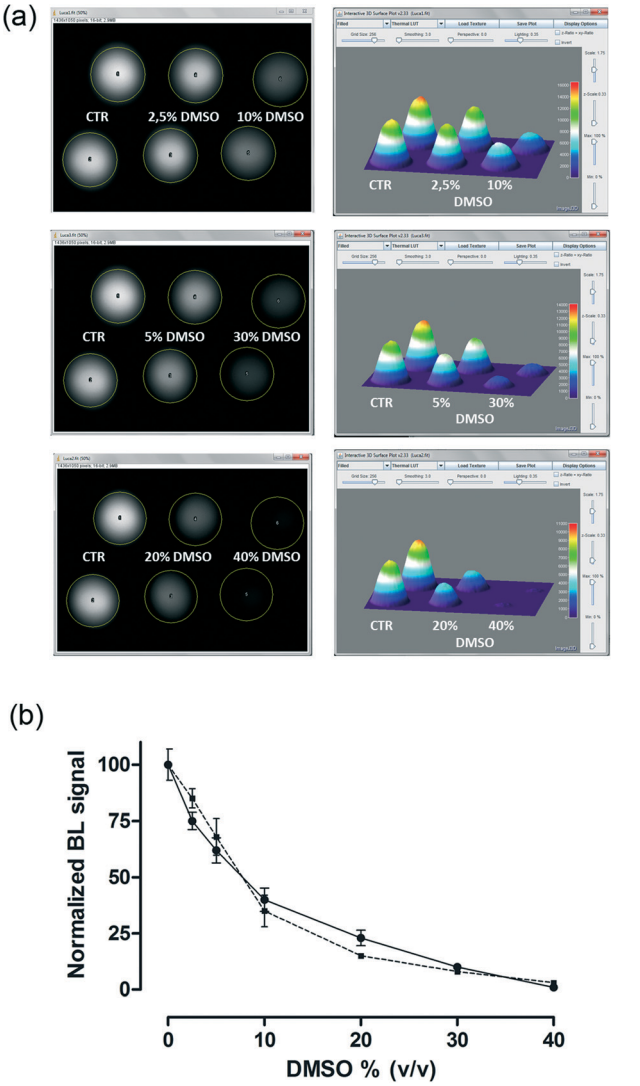

Fig. 4 (a) BL images obtained with the MAGNETOX and 3D surface plot visualization using ImageJ software. Circular ROI were selected and the BL signals were quantified. (b) Normalized toxicity curves for DMSO (30 min incubation) obtained with the BL-MTB in the microwell cartridge (dashed line) and with the MAGNETOX chip (solid line).

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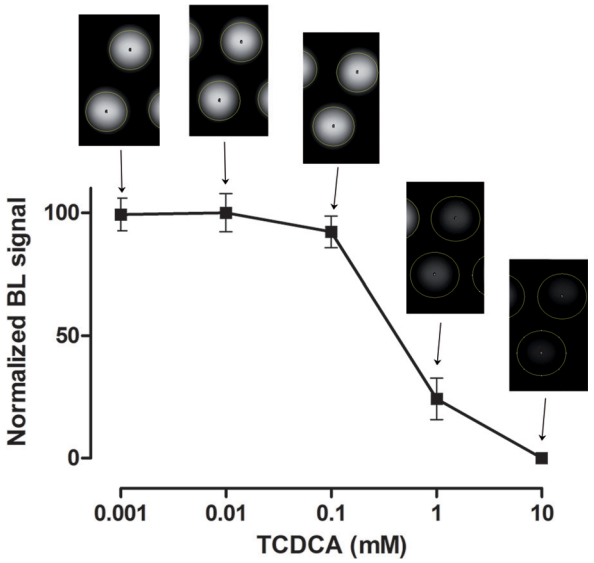

Fig. 5 Toxicity curve for TCDCA (0.001–10 mM) obtained with the MAGNETOX chip. BL images corresponding to each duplicate are shown in the inset. The BL-MTB cells are incubated for 30 min at room temperature with increasing concentrations of TCDCA inside the MAGNETOX chip. After incubation the cells are moved towards the detection chamber, and trapped by applying the magnetic array. Upon addition of the D-luciferin substrate, images are acquired with the CCD sensor and analyzed to quantify BL emission.

The response measured by the MAGNETOX assay was quite reproducible with an intra-assay variability of 15% calculated by considering the 6 detection chambers as replicates and an inter-assay variability of 18% with four replicates. We also compared the results with the closest widely recognized assay, the Microtox® system, which measures the light output of luminescent bacteria (*Vibrio fischeri*) after they have been exposed to a sample. Using 1 h incubation with standard solutions containing different concentrations of DMSO we observed an LC_{50} of 8.3% v/v DMSO with the first toxic effects appearing at 2.5% v/v DMSO (BL signal 81 ± 9%) and complete cell death at DMSO concentrations higher than 40% v/v, (see Fig. S2†), these results are consistent with the MAGNETOX assay.

These preliminary results show that it is possible to exploit magnetic concentration to increase light output and reduce assay volume. Despite this, the reproducibility of the MAGNETOX assay could be improved by optimizing the microfluidic chip design and fabrication. These results support the use of BL-MTB as a powerful tool suitable for microfluidic (bio)sensors.

In addition, the generation of BL-MTB with a codon-optimized version of the luciferase integrated into the bacterial chromosome would surely result in a more robust biosensor. The use of a codon optimized luciferase coding sequence could reduce the time required for its expression. Indeed MAGNETOX assays performed using overnight and 36 h-old cultures resulted in lower analytical performance (*e.g.*, no significant bioluminescent signal at concentrations higher than 20% DMSO v/v and an increased coefficient of variation (CV% = 20%)).

To circumvent this limitation, lyophilized BL-MTB will be obtained providing a ready-to-use suspension of organisms for use in the chip. The implementation of BL-MTB in field-deployable devices could be exploited for direct analysis of environmental or clinical samples containing matrix components, which may interfere with the BL detection but could be easily removed from detection areas. As an alternative, a microelectromagnetic pad actuator could be used to precisely control the movement and positioning of BL-MTB within the microfluidic chip and further miniaturize the system.

Experimental

Chemicals and reagents

All chemicals used for cell culture media preparation and toxic compounds were purchased from Sigma (St. Louis, Missouri, USA). The enzymes required for cloning were from Fermentas (Vilnius, Lithuania). The kits for plasmid extraction and purification were from Qiagen (Hilden, Germany). Sylgard 184 (Dow Corning, USA) was used to create the PDMS chip. D-Luciferin solution, 1 mM at pH 5.0, was prepared by dissolving 28.3 mg D-luciferin sodium salt (Synchem, Kassel, Germany) in 35 mL of 0.1 M citric acid and 65 mL of 0.1 M trisodium citrate solution. Taurochenodeoxycholic acid sodium salt (TCDCA) dilutions were prepared in FSM medium.

Organism and growth conditions

The *M. gryphiswaldense* strain (MSR-1 R3/S1; Rifr Smr spontaneous mutant)[28] was cultured at 28 °C in 10 mL hungate tubes (GPE Scientific UK) in microaerobic conditions (1% O_2 in the headspace). The oxygen concentration in the gas phase was reduced to less than 1% O_2 by repeated flushing with N_2. An MSR-1 medium with 50 μM Fe(III) citrate was used as described by Heyen and Schüler.[29]

Obtainment of bioluminescent magnetotactic bacteria (BL-MTB) and characterization of emission properties

M. gryphiswaldense strain was genetically engineered to constitutively express the red-emitting click beetle luciferase (CBR, λ_{max} = 615 nm). Briefly, the cDNA encoding for CBR was PCR amplified from the vector pCBRbasic (Promega, WI, USA) using the primers CBR Fw AGT**GGATCC**TTACTAACCGCCGGCCTT and CBR Rev CAG**CATATG**GTAAAGCGTGAGAAAAAT, adding BamHI and NdeI restriction sites, shown in bold. These restriction enzymes were then used to digest and insert the luciferases into the pAP150 vector under the control of the $P_{mamDC45}$ constitutive promoter. The resulting vector pAP150-CBR was used to transform the *E. coli* donor strain (BW29427 [thrB1004 pro thi rpsL hsdS lacZ-M15 RP4-1360-(araBAD)567-dapA1341::(erm-pir-)] (K. Datsenko and B. L. Wanner, unpublished) *via* heat shock[30] and transferred to the MSR strain by conjugation.[28] The obtained strain (MSR-CBR) was routinely grown microaerobically at 28 °C in a selective MSR medium containing 5 μg mL^{-1} kanamycin. MSR-CBR

emission kinetics and bioluminescence emission spectra were obtained in a 96-well plate using 100 μL overnight liquid cultures; the BL signal was acquired for 10 minutes (300 ms integration time) with a Varioskan Flash spectral scanning multimode reader (Thermo Scientific, Whaltham, MA, USA) after automatic injection of 100 μL D-luciferin 1 mM, pH 5.0. Bandwidths (nm) of the emission spectra were measured at 50 and 20% of the intensity at the maximum wavelength. Data were analyzed with GraphPad Prism v5.02 (Software, Inc., San Diego, CA, USA). All light measurements were performed in triplicate.

TEM micrographs of MSR-1 expressing CBR luciferase were taken at 18k × and 71k × with a Morgagni 268 at 80 kV.

Evaluation of luciferase time dependence and magnetic orientation

The magnetic orientation (C_{mag}) of the MSR-CBR cells was evaluated spectrophotometrically using the optical density at 565 nm as previously described.[31] Briefly, at given time intervals, cell suspensions of 1.0 mL were withdrawn from the culture for C_{mag} measurement. Cell density was set to an OD of 0.1 and an external magnetic field was applied to align the cells at different angles towards the light beam of the spectrometer. This results in maximum and minimum light extinction and the ratio of these correlates with the average number of magnetosomes per cell. This method is used to semi-quantitatively assess the magnetism of a culture (non-magnetic cells have a C_{mag} value of 0).

Design and fabrication of the MAGNETOX microfluidic chip

The microfluidic chip fabrication process is based on multiple layer casting of black and transparent PDMS on a home-made master mold.

Transparent PDMS was prepared using a monomer curing agent in a weight ratio of 5 : 1, 25 mg mL^{-1} of activated charcoal powder was added to obtain black PDMS. The solutions were then centrifuged at 4000 rpm for 10 min to remove bubbles and stored at −20 °C until use.

Black PDMS was first poured in to fill up to the edge of the relief structures on the mask, which creates the diamond-shaped chambers (7.0 × 14.0 mm diagonals, height 1.5 mm), the microfluidics channels (1 mm width) and detection areas (3 mm diameter, height 1.5 mm). To avoid mixing with transparent PDMS, black PDMS was allowed to harden for 1 h at 60 °C; then a thin layer of transparent PDMS was poured on top of the black PDMS layer, to create the transparent bottom of the wells, and allowed to harden for 2 h at 60 °C. During the curing process the black and transparent PDMS layers fuse together.

A separate layer of transparent PDMS was cast in a different mold, comprising inlets and outlets, to create the top of the chip and allowed to harden as previously described. The two partially-cured PDMS layers were then removed from their masks, superimposed and hardened overnight at 70 °C to obtain the final chip (see Fig. 2(a)).

An array of neodymium–iron–boron circular disc magnets (NdFeB; NeoDeltaMagnet NE32, 3 mm diameter, L = 2 mm, remanence 1170–1250 mT, IBS Magnet, Berlin, Germany) was placed over the detection chambers of the PDMS chip.

The MAGNETOX device consists of the microfluidic device connected to a CCD camera modified for lens-free CL imaging detection. The CCD imaging detector was built from a MZ-2PRO CCD camera (MagZero, Pordenone, Italy) equipped with a Sony ICX285 monochrome CCD image sensor (1360 × 1024 pixels, pixel size 6.45 × 6.45 μm^2) and a 16 bit analog-to-digital (A/D) converter. To reduce thermal noise, the CCD sensor was thermoelectrically cooled by a double Peltier cell. A round fiber optic taper (25/11 mm size, Edmund Optics, Barrington, NJ) was placed in contact with the CCD sensor as previously described by our group.[22] The MAGNETOX is computer controlled *via* a USB 2.0 interface using software (EZ Cap, v3.13) that facilitates data acquisition and parameter settings.

Design of a microwell cartridge

A custom made PDMS 6-well cartridge was produced using black and transparent PDMS as previously described. The mask for PDMS casting has been designed in order to obtain an array of 2 × 3 wells of 4 mm diameter and 4.5 mm deep each.

First, black PDMS was poured in to fill up to the edge of the mask followed by addition of a thin (<200 μm) layer of transparent PDMS to create the bottom of the wells (see Fig. S1(b)†). After overnight incubation at 70 °C the cartridge was carefully separated from the mask.

Toxicity evaluation using the MAGNETOX platform

Different concentrations of dimethyl sulfoxide (DMSO) (in the range 2–50% v/v) and TCDCA (in the range 0.001–10 mM) were used as model toxic compounds to evaluate the feasibility of using BL magnetotactic bacteria as a toxicity biosensor. All serial dilutions of compounds were performed using FSM medium as a diluent.

Different experimental conditions were optimized to improve the biosensor performance (*e.g.*, incubation temperature and time, volumes and ratio of cell suspension to sample). Under optimized conditions, an analysis with the MAGNETOX chip includes the following steps: i) 40 μL of 3 day-old liquid culture is driven into the chip by an air-displacement pipette; ii) cells are incubated for 30 min at room temperature with 20 μL of sample (medium is used as blank); iv) cells are moved and concentrated in the detection chambers by adding the array of permanent magnets; v) BL imaging measurements after addition of 1 mM D-luciferin pH 5.0. Images are acquired for 5 min and analyzed with ImageJ software v.1.46 (National Institutes of Health, Bethesda, MD). Images are recorded in the FITS (Flexible Image Transport System) format. Regions of interest (ROIs) corresponding to detection chambers are selected and light emissions quantified as raw integrated densities.

For toxicity experiments, normalized BL signals (the BL emission of the untreated control was set as 100%) were plotted against toxic compound concentration. The lethal concentration (LC_{50}) of each compound was calculated as the concentration producing a 50% reduction in light.

All experiments were performed in duplicate and repeated at least three times.

The toxic effects of DMSO and TCDCA solutions (in the range 2–50% v/v and 0.001–10 mM, respectively) were also assessed using a Microtox® assay with *Vibrio fischeri*.[32] Different exposure times were tested (10, 30 and 60 min at 25 °C) in 96 microplate format using 90 μL of cell suspension and 10 μL of analyte or control (medium). The results were analyzed as described for the MAGNETOX assay.

Conclusions

Here, for the first time, the use of bioengineered bioluminescent magnetotactic bacteria in combination with microfabrication technologies is reported for biosensing applications. The novel concept of a black and transparent PDMS microfluidic chip has been developed which could find broad use in the optofluidic field. The chip has been integrated with a portable CCD sensor for lens-less imaging detection of light signals emitted by the BL magnetotactic bacteria used as biosensing "living actuators". Unlike other whole-cell biosensors, BL-MTB can be easily moved and concentrated in specific detection chambers, where the sample matrix is removed and bacteria are washed, thus increasing the analytical signal and performance of the system. The interaction of BL-MTB with the analyte is facilitated in the detection chamber since this interaction takes place in a dispersed suspension, resulting in a shorter incubation time. In this regard, the MAGNETOX assay faciliates rapid (30 min) measurement of sample toxicity with the non negligible advantage of chip re-usability.

This is the first attempt to integrate bioengineered magnetotactic bacteria into an analytical device and several optimizations regarding both the cell and the chip design will be addressed. An array of electromagnets or a microelectromagnetic pad actuator will be included to better control the BL-MTB within the chip by tuning the magnetic trapping or continuously directing their swimming to the detection area.

Although many improvements are required before applying BL magnetic biosensors to real-life needs, we are confident that they represent the forerunner of a new concept in whole-cell biosensing.

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